

**ENCAPSULATION OF THERAPEUTIC PROTEIN WITHIN POLYMERIC  
NANOFIBER USING CO-AXIAL ELECTROSPINNING**

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## ABSTRACT

A drug delivery system is designed to provide a therapeutic agent in the needed amount, at the right time and to the proper location in the body in a manner that optimizes the efficacy, increases compliance and minimizes side effects. In order to study the encapsulation of therapeutic protein within polymeric nanofiber for controlled release using co-axial electrospinning method, a number of main processing parameters were taken into considerations which are formulation of drug loading, polymer and protein concentration and solution flow rate. Polymeric drug delivery device was developed via electrospinning technique using biodegradable Polymer A. Co-axial electrospinning configuration was used to encapsulate various mixtures of Drug 0066, Drug 0360 and also Polymer B as support into the electrospun nanofibers. Using the configuration, two separate solutions flowed through two different capillaries and electrospun through co-axial nozzle configuration setup. The electrified jet will undergo stretching, leading to formation of long and thin thread. When the liquid jet is continuously elongated, the solvent will evaporate. The grounded collector will attract the charged fiber. The morphology of the electrospun nanofibers were analyzed using Field Emission Scanning Electron Microscopy (FE-SEM) and Transmission Electron Microscopy (TEM). The hydrophilicity of electrospun nanofibers were determined using Surface Contact Angle machine. Fourier Transform Infrared Spectrometry (FT-IR) was used to detect the organic group of the electrospun nanofibers. In vitro release studies were conducted to evaluate sustained release potential of the core-sheath structure composite nanofiber. The results showed that the TEM images clearly proved the core/shell structure of nanofibers for the encapsulation of Drug 0066/Drug 0350 within Polymer A. SEM also showed there was an arch appeared within the nanofiber. The present study would provide a basis for further design and optimization of processing conditions to control the nanostructure of core-sheath composite nanofibers and ultimately achieve desired release kinetics of bioactive proteins (e.g., growth factors) for practical tissue engineering applications.

## ABSTRAK

Sistem penghantaran dadah direka untuk menyediakan agen terapeutik dalam jumlah yang diperlukan, pada masa yang tepat dan lokasi yang sepatutnya di dalam badan dengan cara yang mengoptimalkan keberkesanan, meningkatkan pematuhan dan mengurangkan kesan sampingan. Dalam usaha untuk mengkaji pengkapsulan protein terapeutik ke dalam nanofiber berpolimer untuk pelepasan terkawal dengan menggunakan *electrospinning* sepaksi, beberapa parameter utama pemprosesan telah diambil kira untuk formulasi muatan dadah, kepekatan polimer dan protein dan kadar aliran cecair. Peranti penghantaran dadah berpolimerik telah dibangunkan melalui teknik *electrospinning* menggunakan Polimer A yang mudah terurai. Konfigurasi *electrospinning* sepaksi digunakan untuk mengurung pelbagai campuran Dadah 0066, Dadah 0350 dan juga Polimer B sebagai sokongan kepada nanofiber. Menggunakan konfigurasi ini, dua cecair yang berasingan mengalir melalui dua kapilari yang berlainan dan melalui konfigurasi muncung berpaksi *electrospinning*. Jet elektrik akan menjalani regangan, membawa kepada pembentukan benang yang panjang dan nipis. Apabila jet cecair memanjang secara berterusan, pelarut akan menguap. Alat pengumpul akan menarik serat yang bercas. Morfologi nanofibers dianalisis menggunakan *Field Emission Scanning Electron Microscopy* (FE-SEM) dan *Transmission Electron Microscopy* (TEM). Hydrophilicity nanofiber telah ditentukan menggunakan mesin *Surface Contact Angle*. *Fourier Transform Infrared* Spektrometri (FT-IR) telah digunakan untuk mengesan kumpulan organik nanofiber. Kajian pembebasan *In vitro* telah dijalankan untuk menilai potensi pembebasan dadah di dalam struktur komposit nanofiber. Hasil kajian menunjukkan bahawa imej-imej TEM jelas membuktikan struktur teras/luar nanofiber bagi pengkapsulan Dadah 0066/Dadah 0350 di dalam Polimer A. SEM juga menunjukkan terdapat timbunan muncul dalam nanofiber. Kajian ini akan menyediakan asas bagi reka bentuk dan mengoptimalkan keadaan pemprosesan bagi mengawal struktur nanofibers komposit teras-luar dan akhirnya mencapai kinetik pelepasan yang diinginkan protein bioaktif (contohnya, faktor pertumbuhan) untuk aplikasi secara praktikal dalam kejuruteraan tisu.

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## LIST OF SYMBOLS/ABBREVIATIONS

°C	A scale and unit of measurement for temperature
%	Percent
μL	Microliter
μm	Micrometer
cm	Centimeter
g	Gram
kV	Kilovolts
M	Molar
mL	Milliliter
mL/h	Volumetric flow rate
mm/min	Measurement of flow
h	Hour
mol/L	Molar concentration
MW	Molecular weight
nm	Nanometer
wt %	Mass fraction
w/v %	Mass concentration
FE-SEM	Field Emission Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
FT-IR	Fourier Transform Infrared Spectrometry
DMF	Dimethylformamide
PBS	Phosphate buffer saline
TFE	2, 2, 2-Trifluoroethanol
NUSNNI	National University of Singapore Nanotechnology & Nanoscience Initiative
GMP	Good Manufacturing Practise
FDA	Food and Drug Administration

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of Study

Nanotechnology by manipulation of characteristics of materials such as polymers and fabrication of nanostructures is able to provide superior drug delivery systems for better management and treatment of diseases. Benito (2006) states, basically, the concept behind drug delivery is to provide more constant concentrations in the organism, and to bring the compound with pharmaceutical activity directly to the site of need in order to enhance the effectiveness of action.

According to Kim and Pack (2006), a wide variety of new, more potent and specific therapeutics are being created in advances in biotechnology. A drug delivery system is designed to provide a therapeutic agent in the needed amount, at the right time and to the proper location in the body in a manner that optimizes the efficacy, increases compliance and minimizes side effects. Due to common problems in drug delivery such as low solubility, high potency and poor stability, it can impact the efficacy and potential of the drug itself. Thus, there is a corresponding need for safer and more effective methods and devices for drug delivery.

One way to bring the active substance to the site of action is to modify their bio-distribution by entrapping them in particulate drug carriers (Benito, 2006). By encapsulating drugs in designed carriers, labile drugs are protected from degradation inside the hostile conditions. Within the concept of drug delivery the mechanism must be taken

into account to design such carrier systems is sustained or controlled the drug delivery. Controlled-release is aimed at obtaining enhanced effectiveness of the therapeutic treatment by minimizing both under- and over-dosing. A frequently desired feature is to achieve a constant level of drug concentration in the blood circulation or at the site of action of the substance, with a minimum of intakes per day and a maximum coverage. Usually drug delivery systems that dissolve, degrade, or are readily eliminated are preferred.

Biodegradable polymers are of great interest since these materials are processed within the body under biological conditions giving degraded sub-units that are easily eliminated by the normal pathways of excretion (Brannon, 1995). According to Yih *et al.* (2006), polymeric nanoparticles are colloidal solid particles with a size range of 10 to 1000nm and they can be spherical, branched or shell structures. Polymeric vesicles could provide a protective environment for protein molecule to deliver them intact to desired targets.

Among many approaches of fabricating nanofibers, electrospinning, which is also known as electrostatic spinning, is perhaps the most versatile process. This technique allows for the production of polymer fibers with diameters varying from 3 nm to greater than 5  $\mu\text{m}$  (Pham *et al.*, 2006). Moreover, it can easily fabricate nanofiber and microfiber meshes from different types of polymer. Due to their unique features such as high surface-to-volume ratio, morphological design flexibility and extracellular matrices structure-like, nanofibers are used as scaffolds for drug delivery and tissue engineering. Low molecular weight drugs and biomolecules such as proteins and nucleic acids can be encapsulated into the electrospun fibers (Xu *et al.*, 2008).

## 1.2 Problem Statement

Developing protein and peptide-based drugs present challenges to drug delivery scientists because of their unique nature and difficulty in delivery through conventional routes. The delivery of these therapeutic proteins is limited by their fragile structure and frequent monitoring required. Releasing a protein without denaturation when the polymer is

degraded is what the researcher concern about. When protein is released over time, protein instability problems may occur and result in incomplete release even when the polymer has been degraded. Previous studies shown that the co-axial electrospinning gives an impressive successful method to ensure the bioactivity of these proteins is retained. Coaxial electrospinning was developed for simultaneously electrospinning two different polymer solutions into core/shell nanofibers, or encapsulated bioactive molecular and drugs into polymer nanofibers for controlled release (Chen *et al.*, 2010). Varying the processing parameters will effect to the diameter size and protein release profile of the polymeric drug delivery. By investigating this polymeric drug delivery system, it would able to improve therapeutic efficacy by releasing protein at a controlled rate over a period of time.

### 1.3 Research Objectives

In this study, there are two objectives aligned to achieve the purpose of encapsulation of therapeutic protein within polymeric nanofiber for controlled release using co-axial electrospinning. The objectives are to develop a polymeric drug delivery system using electrospun nanofibers and to characterize the electrospun nanofibers with various mixtures of drugs encapsulation.

### 1.4 Scope of Study

The study covers of development of a polymeric drug delivery system using electrospun nanofibers and to characterize the electrospun nanofibers with various mixtures of drugs encapsulation. The scope of study of this experiment is categorized to experimental design and parameters evaluation. The design of this experiment is based on co-axial electrospinning setups and mechanisms. Parameters evaluation of this study have been identified after considering these aspects as main limitations – polymer and protein concentration, solution flow rate, voltage supply and distance between nozzle's tip and collector. The morphology of the electrospun nanofibers were analyzed using Field Emission Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM). The hydrophilicity of electrospun nanofibers were determined using Surface

Contact Angle machine. Fourier Transform Infrared Spectrometry (FTIR) was used to detect the organic group of the electrospun nanofibers. In vitro release studies were conducted to evaluate sustained release potential of the core-sheath structure composite nanofiber.

## 1.5 Rationale and Significance

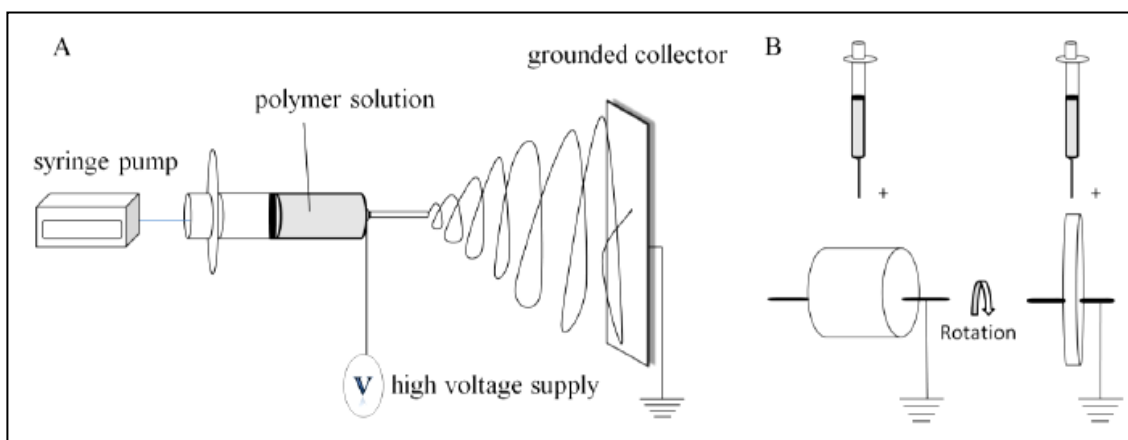
Therapeutic proteins are one of the most important and rapidly growing segments of the pharmaceutical market, with estimated annual world-wide sales of over \$35 billion in 2005 (Martin, 2006). The potential of these electrospun nanofibers in healthcare application is promising, for example as vector to deliver drugs and therapeutics. Electrospun fiber mat provide the advantage of increased drug release due to the increased surface area. According to Jiang *et al.* (2005), the interconnected, three-dimensional porous structure and enormous surface area of electrospun nanofibers prepared from biodegradable polymers have great potential in tissue engineering, drug delivery and gene therapy. This is due to their biodegradability and fiber-forming properties. The significance of this study is the production of polymer nanofiber membranes encapsulated with therapeutic proteins. It is found that using coaxial nozzle configuration in electrospinning, water-soluble therapeutic proteins can be encapsulated into biodegradable non-woven polymer fibers resulted in subsequent controlled release compared with other methods. Encapsulation of protein using electrospun nanofibers has the advantages of being facile, high loading capacity and efficiency, mild preparation condition and steady release characteristics (Jiang *et al.*, 2005).

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Processes of Electrospinning

According to Lu *et al.* (2008), the process of electrospinning is based on the principle that strong electrical force overcomes weaker surface tension of a polymer solution at certain threshold to eject a liquid jet, could trace its root back to the process of electrospray in which small solid polymer droplets are formed. It is a variation of the electrostatic spraying process where high voltage induces the formation of a liquid jet (Rao, 2009). A typical electrospinning setup consists of three major components which are a high voltage power supplier, a syringe with a metal needle connected to a syringe pump, and a grounded conductive collector (Figure 2.1(A)). A polymer solution is loaded into the syringe and the desired flow rate is controlled by the syringe pump.



**Figure 2.1:** Schematic of the electrospinning process. (A) a typical electrospinning set-up and (B) collection methods for creating aligned fibrous scaffolds using rotating drum and rotating disk.

Source: Lee *et al.* 2011

In the electrospinning process, a high voltage is applied to a droplet formed from a polymer solution or melt at the tip of the metal needle. Li *et al.* (2004) explains that the high voltage applied on the nozzle or the needle containing the polymer drop causes it to get highly electrified and the charges are distributed along the surface of the drop evenly. There are two types of electrostatic forces that the drop experiences, namely electrostatic repulsion (b/w surface charges) and the Coulombic force (exerted by external electric field). Doshi and Renekar (1995) explain that these charges undergo mutual repulsion that causes a force which is directly opposite to the surface tension. When the electric field is intensified, elongation of the hemispherical surface of the solution present at the tip of the needle occurs resulting in the formation of a conical shaped structure called as the Taylor cone.

The charging of the fluid leads to the formation of a Taylor cone of the droplet and eventually to the ejection of a liquid jet from the apex of the cone once the strength of electric field has surpassed a certain threshold value. The electrified liquid jet is accelerated



towards the grounded collector by the electric field and thins rapidly due to the evaporation of the solvent and elongation by stretching and whipping. The solidified fiber is often deposited as a randomly oriented, nonwoven mat of nanofibers (Li *et al.*, 2004).

Huang *et al.* (2003) states that further increasing the electric field, a critical value is attained with which the repulsive electrostatic force overcomes the surface tension and the charged jet of the fluid is ejected from the tip of the Taylor cone. The discharged polymer solution jet undergoes an instability and elongation process, which allows the jet to become very long and thin. Meanwhile, the solvent evaporates, leaving behind a charged polymer fiber. In the case of the melt the discharged jet solidifies when it travels in the air.

Based on a review conducted by Pham *et al.* (2006), the shape of the base depends upon the surface tension of the liquid and the force of the electric field; jets can be ejected from surfaces that are essentially flat if the electric field is strong enough. Charging of the jet occurs at the base, with solutions of higher conductivity being more conducive to jet formation. Lee and Arinzeh (2011) justify that the most common method to collect the electrospun nanofibers is on a high speed rotating drum or disk (Figure 2.1(B)). This allows for the fiber to collect along the direction of rotation. Small diameter tubes can also be fabricated by this method and have been used in vascular repair studies. A high rotation speed produces increased fiber alignment as compared to lower rotation speed, but may cause fiber discontinuity.

## 2.2 General Set-Ups and Processing Parameters

Electrospinning is an efficient, inexpensive technique in which the whole apparatus is compact. The basic set up is a syringe with a metal needle connected to a syringe pump, grounded collector, and a high voltage source. Over the years researchers have found the need to modify the set up for various reasons, but the basic principle has been the same.

Lu *et al.* (2008) states that although the setup for electrospinning is extremely simple, the detailed experimental and theoretical analysis reveals that the electrospinning

process is highly complex. Doshi and Renekar (1995) explain that many parameters can influence the transformation of polymer solution into nanofibers through electrospinning. These parameters include (a) the solution properties such as viscosity, elasticity, conductivity, and surface tension, (b) governing variables such as hydrostatic pressure in the capillary tip, and the gap (distance between the tip and the collecting screen), and (c) ambient parameters such as solution temperature, humidity, and air velocity in the electrospinning chamber.

### 2.2.1 Needle Diameter (Nozzle)

Rao (2009) elaborates that in electrospinning, a precise amount of polymer solution is taken in the capillary or spinneret. The nozzle (usually the syringe needle set up) determines the amount of polymer melt that comes out, which in turn affects the size of the drop being formed and also the pressure or the amount of force required by the pump so as to push the melt out. If the polymer melt is less viscous, then it can easily come out of the nozzle. The polymer melt is usually a thick highly viscous fluid. So, if the nozzle is too small, then unless it's less viscous, the melt cannot be forced out. Hence, an appropriate nozzle should be chosen. Different types of nozzles or spinnerets have been used over the years. Warner *et al.* (1999) used a spinneret which was basically a stainless steel tube with an outer diameter of 1/16th inch and inner diameter of 0.04 inch. They have also used a capillary of 1.6mm in their experiments.

According to Mo *et al.* (2004), the internal diameter of the needle of the pipette orifice has a certain effect on the electrospinning process. A smaller internal diameter was found to reduce the clogging as well as the amount of beads on the electrospun fibers. The reduction in the clogging could be due to less exposure of the solution to the atmosphere during electrospinning. Decrease in the internal diameter of the orifice was also found to cause a reduction in the diameter of the electrospun fibers. When the size of the droplet at the tip of the orifice is decreased, the surface tension of the droplet increases. Zhao *et al.* (2004) argues that if the diameter of the orifice is too small, it may not be possible to extrude a droplet of solution at the tip of the orifice.

### 2.2.2 Distance between Tip and Collector

Sill *et al.* (2008) states that the distance between capillary tip and collector can also influence fiber size by 1-2 orders of magnitude. Additionally, this distance can dictate whether the end result is electrospinning or electrospraying. Doshi and Reneker found that the fiber diameter decreased with increasing distances from the Taylor cone. In another study, Jaegar *et al.* (1998) electrospun fibers from a PEO/water solution and examined the fiber diameter as a function of the distance from the Taylor cone. They found that the diameter of the fiber jet decreased approximately 2-fold, from 19 to 9  $\mu\text{m}$  after travelling distances of 1 and 3.5cm, respectively.

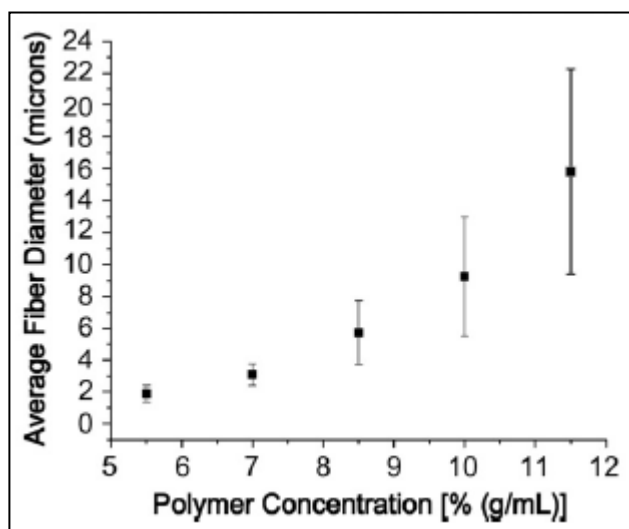
The distance between the tip and the collector will have a direct influence in flight time and electric field strength. For fibers to form, the electrospinning jet must be allowed time for most of the solvents to be evaporated. When the distance between the tip and the collector is reduced, the jet will have shorter distance to travel before it reaches the collector plate. The electric field strength will increase at the same time and this will increase the acceleration of the jet to the collector. As a result, there may not have enough time for solvents to evaporate when it reach the collector. When the distance is too low, excess solvents may cause the fibers to merge when they contact to form junctions resulting in intra layer bonding (Ramakrishna *et al.*, 2005).

In a study constructed by Dietzel *et al.* (2001), they had a needle to collector distance of about 20cm while in Warner *et al.* (1999) study, they had a distance of 15cm. Subbiah *et al.* (2004) explains that morphology of the electrospun fibers depends on the evaporation rate, deposition time, and whipping interval. If the distance is too small, it would result in collection of wet fibers and fibers having a bead-like structure. Hence, a suitable distance should be set so that the fibers have enough time to dry.

### 2.2.3 Polymer Concentration

Sill *et al.* (2008) justifies that polymer concentration determines the spinnability of a solution. The solution must have high enough polymer concentration for chain entanglements to occur. However, the solution cannot be either too dilute or too concentrated. The polymer concentration influences both the viscosity and surface tension of the solution. If the solution is too dilute, the polymer fiber will break up into droplets before reaching the collector due to the effect of surface tension. If the solution is too concentrated, then fibers cannot be formed due to the high viscosity which makes it difficult to control the solution flow rate through the capillary. An optimum range of polymer concentration exists in which fibers can be electrospun when all other parameters are held constant. Figure 2.2 shows that the mean fiber diameter increases monotonically with increasing polymer concentration.

On the other hand, Doshi and Reneker had electrospun fibers from PEO/water solutions containing various PEO concentrations and found that solution with viscosity less than 800 centipoises broke up into droplets upon electrospinning while solutions with viscosity greater than 4000 centipoises were too thick to electrospin. In many experiments it has been shown that within the optimal range of polymer concentrations fiber diameter increases with increasing polymer concentration. Deitzel *et al.* found that fiber diameter of fibers electrospun from PEO/water solution were related to PEO concentration by a power law relationship.



**Figure 2.2:** The relationship between the average fiber diameter and the polymer concentration is given. Note that the mean fiber diameter increases monotonically with increasing polymer concentration.

Source: Sill *et al.* (2008)

#### 2.2.4 Solution Flow Rate (Mono-Axial Electrospinning)

According to Sill *et al.* (2008), polymer flow rate also has an impact on fiber size, and additionally can influence fiber porosity as well as fiber shape. They state that the cone shape at the tip of the capillary cannot be maintained if the flow of solution through the capillary is insufficient to replace the solution ejected as the fiber jet. Megelski *et al.* examined the effects of flow rate on the structure of electrospun fibers from a polystyrene/tetrahydrofuran (THF) solution. They demonstrated that both fiber diameter and pore size increase with increasing flow rate. Additionally, at high flow rates significant amounts of bead defects were noticeable, due to the inability of fibers to dry completely before reaching the collector. Incomplete fiber drying also leads to the formation of ribbonlike (or flattened) fibers as compared to fibers with a circular cross section.

According to Ramakrishna *et al.* (2005), the flow rate will determine the amount of solution available for electrospinning. For a given voltage, there is a corresponding feed rate if a stable Taylor cone is to be maintained. When the feed rate is increased, there is a corresponding increase in the fiber diameter or beads size. This is due to greater volume of solution that is ejected from the needle tip. Yuan *et al.* (2004) argues that a lower feed rate is more desirable as the solvent will have more time for evaporation. The jet will take a long time to dry due to the greater volume of solution drawn from the needle tip.

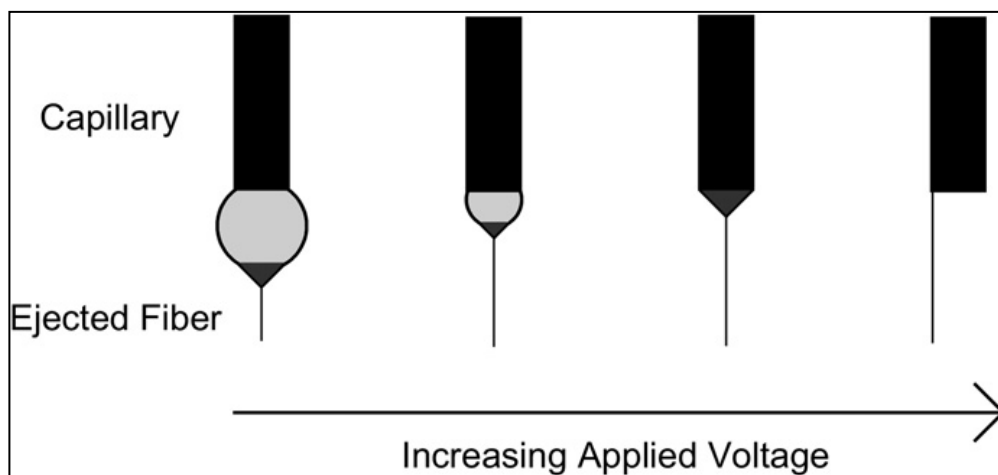
The rate at which the polymer comes out of the needle/nozzle is an important factor in electrospinning. Doshi and Reneker (1993) filled a capillary tube with the polymer solution and a hydrostatic pressure was established by an air pump which was controlled by valves and was read on a manometer. Warner *et al.* (1999) used a digitally controlled, positive displacement syringe pump (Harvard Apparatus PHD 2000) and had typical flow rates ranging between 0.2 ml/min to 1 ml/min. Dietzel *et al.* (2001), used a flow rate of 0.05ml/hr achieved using a Harvard 2000 syringe pump. Subbiah *et al.* (2004) mentioned that the material transfer rate and the jet velocity are directly dependent on this feature. They have also mentioned that researchers have found that the higher the polymer flow rate, bigger the diameter of the fibers.

### 2.2.5 Voltage Supply

One of the most studied parameters among the controlled variables is the effect of field strength or applied voltage. Rao (2009) explains that a suitable high voltage is applied on the needle such that, when it exceeds a critical value, the drop which is induced at the tip of the needle distorts into the shape of a cone and a charged jet of the polymer erupts from the apex of this cone. This jet gets drawn towards the grounded collector by the electric field. Similarly, Warner *et al.* (1999) used a Gamma High Voltage Research ES30-P power supply to induce a voltage up to 20 kV in their experiments. Dietzel *et al.* (2001) found this critical value to be 5 kV. They have applied voltages ranging from 5kV-15kV in their experiments.

According to Pham *et al.* (2006), at low voltages or field strengths, a drop is typically suspended at the needle tip, and a jet will originate from the Taylor cone producing bead-free spinning (assuming that the force of the electric field is sufficient to overcome the surface tension). As the voltage is increased, the volume of the drop at the tip decreases, causing the Taylor cone to recede. The jet originates from the liquid surface within the tip, and more beading is seen. As the voltage is increased further, the jet eventually moves around the edge of the tip, with no visible Taylor cone. At these conditions, the presence of many beads can be observed.

Similarly, Sill *et al.* (2008) explains that at relatively low applied voltages, a pendant drop (depicted in light gray) is formed at the tip of the capillary as shown in Figure 2.3. The Taylor cone (depicted in dark gray) then forms at the tip of the pendant drop. However, as the applied voltage is increased (moving from left to right), the volume of the pendant drop decreases until the Taylor cone is formed at the tip of the capillary. Increasing the applied voltage further results in the fiber jet being ejected from within the capillary that is associated with an increase in bead defects.



**Figure 2.3:** Effect of varying the applied voltage on the formation of the Taylor cone.

Source: Sill *et al.* 2008

### 2.2.6 Humidity

The humidity was varied by Casper *et al.* (2004), while spinning polystyrene solutions. Their work showed that increasing the humidity resulted in the appearance of small circular pores on the surface of the fibers and further increasing the humidity will lead to the pores coalescing as determined by atomic force microscopy. At high humidity, it is likely that water condenses on the fiber surface when electrospinning is carried out under normal atmosphere. As a result, this may have an influence on the fiber morphology especially polymers dissolved in volatile solvents (Megelski *et al.*, 2002). According to him, water vapor may condense on the jet surface due to jet surface cooling as a result of rapid evaporation of the volatile solvent. Pores are created when both water and solvent eventually evaporate. Pores seen on electrospun fibers mat due to the dynamic condition of the electrospinning jet as compared to static condition

The humidity of the environment will also determine the rate of solvent evaporation of the solvent in the solution. At a very low humidity, a volatile solvent may dries very rapidly. The solvent evaporation may be faster than the removal of the solvent from the needle tip. As a result, the electrospinning process may only be carried out for a few minutes before the needle tip is clogged (Ramakrishna *et al.*, 2006).

## 2.3 Co-Axial Electrospinning

In many cases, the application of nanofibers is required to keep the functionalizing agents (for example, biomolecules such as enzymes, proteins, drugs, viruses, and bacteria) in the fluid environment to maintain their functionality. In order to meet this requirement, core-shell nanofibers were prepared by a modified electrospinning process, co-axial electrospinning. According to Yarin (2010) in his review, he mentions that co-axial electrospinning or co-electrospinning of core-shell micro- and nanofibers was born 7 years ago as a branch of nanotechnology which bifurcated from a previously known electrospinning. Through electrospinning, co-electrospinning inherited roots in polymer science and electrohydrodynamics, while some additional genes from textile science and